The Role of the Horizontal Gene Pool and Lateral Gene Transfer in Enhancing Microbial Activities in Marine Sediments

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Project Objective:

Plasmid-encoded traits mobilized in microbial communities can provide rapid adaptation to changing environmental conditions. Presently, we know little of the mobility of plasmid-encoded traits and the factors that regulate horizontal (lateral) gene transfer in the marine environment. The assessment of the role of horizontal gene transfer (HGT) in the evolution of marine sediment bacterial populations and their activities is the primary objective of the project. Primary objectives include: (1) determining the regulation of transfer of mobile genetic elements in marine bacterial communities; and (2) assessing the chemical (e.g., organic and inorganic nutrients, contaminant bioavailability) and physical (e.g., surface availability) characteristics of marine sediments that facilitate HGT by promoting microbial interactions.

Approach:

To investigate plasmid distribution and plasmid-mediated effects on marine microbial community activities, plasmids are obtained from bacterial populations and characterized at the molecular level. DNA probes specific for replication regions (e.g., plasmid incompatibility-group probes) are used to characterize the distribution, diversity and persistence of the replicons in marine environments. Marine plasmids are also being sequenced to determine biological functions. The transfer dynamics of plasmids will be assessed by elucidating environmental and molecular constraints likely to affect horizontal gene exchange. In addition, we have developed new molecular techniques to rapidly assess plasmid populations along spatial and temporal scales.

Accomplishments:

During this project we have relied on previously obtained marine plasmid sequence data to conduct a series of laboratory-based experiments to determine possible ecological roles of selected cryptic marine plasmids. We focused on experimental efforts on a marine *Vibrio* strain denoted 22702. Phylogenetic and biochemical analyses indicated the strain is a *Vibrio parahaemolyticus*. This strain contains two extrachromosomal elements, plasmid 22702B (28.8 Kb) encoding putative functions for

the uptake of exogenous DNA and a smaller replicon 22702A (9.0 Kb). The second element, 22702A exhibits a high degree of homology (i.e., 95-100% identical at the amino acid level) to the previously described prophage p03K6 isolated from *Vibrio parahaemolyticus*. Our results have shown a significant difference between the survival and persistence of *Vibrio* sp. 22702 compared to other marine *Vibrios* when cells are supplied with an exogenous source of DNA. We examined growth and persistence of *Vibrio* spp. 22702 and 09022, a closely related Vibrio strain, at environmentally relevant densities with or without DNA as a sole carbon and energy source. Strain 22702 has enhanced survival and persistence during growth on exogenous DNA as a sole carbon source. We are completing these experiments and will submit a manuscript describing these findings. The ability to survive on alternate carbon sources is an important capability of marine *Vibrios* and has implications with regard to other marine *Vibrio* pathogens.

To better understand plasmid distribution, diversity and abundance in marine sediment microbial communities we developed a number of methodological approaches to advance the field during this project. In addition to replicon typing, a new approach to facilitate the rapid comparison and differentiation of marine plasmids has recently been developed by our group (Beeson *et al.,* 2002). The method is based on the randomly amplified polymorphic DNA (RAPD) method developed for chromosomal characterization. When the method was applied to 100 endogenous plasmids isolated from cultivated marine diazotrophs from salt marsh grass rhizoplane niches remarkably a total of 71 different plasmid genotypes were detected with 57 of the groups containing only one plasmid. These findings are in contrast to the 12 structural groups, as determined by RFLP profiling, previously observed with exogenously isolated marine plasmids. In addition to genotyping marine plasmids, the PCR-based RAPD method shows considerable promise for tracking spatial and temporal changes in marine plasmid populations. This method is applicable to other systems and can be used to type plasmids isolated from diverse microorganisms.

We also continued our efforts optimize large-scale plasmid community extraction and isolation procedures that would provide enriched or purified supercoiled plasmid DNA from bulk marine sediments suitable for the construction of 'plasmid metagenome libraries'. Such libraries would provide access to the unculturable fraction of plasmids resident in marine microbial communities. We applied a new microbial ecology technique, stable isotope probing, as part of our efforts. The coupling of molecular techniques with stable isotope probing (SIP) provides a powerful means by which to ascertain metabolically active microorganisms, especially those not readily cultured. To date, while SIP has been employed for the isolation of chromosomal DNA it has not been applied to the isolation of extrachromosomal elements (i.e., plasmid, prophage and accessory DNAs). Such elements may also contribute to the metabolic activity of the host microbe(s). Using cesium chloride density gradient centrifugation we demonstrated the separation and isolation of ^{13}C plasmid DNA from ^{12}C plasmid DNA. In addition, we demonstrated the application of the SIP method to the isolation of plasmid DNA in metabolically active cells using a variety of plasmid-bearing microorganisms, including an indigenous marine bacterium, *Vibrio* sp. strain 22702. Application of SIP-based methods to indigenous microbial assemblages in marine water and sediment systems will allow one to examine not only the prevalence of microorganisms but to identify extrachromosomal elements that are co-labeled during substrate utilization. We are continuing to optimize this method to improve recovery and detection of plasmid molecules. The third methodological advance, conducted in collaboration with Dr. Peter Agron, resulted from our ONR-funded research on marine plasmids was an approach that permits any circular plasmid to be established in *Escherichia coli* (Agron *et al.,* 2002).

Conclusions:

By promoting the movement of genes throughout bacterial populations, plasmids can exert a direct effect on ecological processes. Presently, additional basic information on the molecular functions (i.e., transfer, maintenance, host range, replication and incompatibility) of indigenous plasmids is needed to assess the role of *in situ* plasmid-mediated gene exchange in marine bacterial populations. Continued efforts to sequence marine plasmids and to identify and characterize plasmid distribution and diversity in marine ecosystems will continue to provide new insights and understanding of bacterial gene flux mediated by marine plasmids.

Significance:

The importance of horizontal gene transfer to microbial evolution and adaptation is well recognized. Surprisingly, until recently few studies addressed the role of plasmids in marine environments and their contributions to the activities of marine microorganisms. Understanding the biology and ecology of marine plasmids will provide greater insights into the significance of horizontal gene transfer in marine microbial communities. Such insights can be used to promote, enhance and potentially direct microbial activities in marine environments.

Patent Information: None to report

Award Information: During this award period the principal investigator was tenured and promoted to the level of Associate Professor. She also serves as Editor for the journal *FEMS Microbiology Ecology* and on the editorial board of the journal *Applied and Environmental Microbiology*.

Publications and Abstracts:

(1). Hazen, T.H., K. D. Kennedy, M.S. Humphrys, and P.A. Sobecky. 2006. Characterizing the survival mechanism of a *Vibrio parahaemolyticus* environmental isolate. Meeting Abstract, 106th General Meeting of the American Society for Microbiology.

(2). Hazen, T. H., M.S. Humphrys, K.D. Kennedy, and P.A. Sobecky. 2006. The role of extracellular DNA in carbon starvation adaptation of *Vibrio parahaemolyticus* strains. Meeting Abstract, American Society for Limnology and Oceanography.

(3). Hazen,T.H., M.S. Humphrys, S. Turner, A. Whiteley, M.J. Bailey, and P.A. Sobecky. 2005. Using stable isotope probing to assess plasmid-mediated DNA uptake. Meeting Abstract, Harold Nations Symposium, Georgia Institute of Technology.

(4). Hazen,T.H., M.S. Humphrys, S. Turner, A. Whiteley, M.J. Bailey, and P.A. Sobecky. 2005. Using stable isotope probing to assess plasmid-mediated DNA uptake. Meeting Abstract, 105th General Meeting of the American Society for Microbiology.

(5). Haghshenas, N., J.D. Criminger, G.Y. Matsui, P.A. Sobecky and C.R. Lovell. 2005. *nifH* encoding plasmids of diazotrophic bacteria isolated from roots of a salt marsh grass. Meeting Abstract, 105th General Meeting of the American Society for Microbiology.

(6). Martinez, R.M., T.H. Hazen, A.D. Dugan, M.S. Humphrys, H.J. Mills, and P.A. Sobecky. 2004. The role of a cryptic marine plasmid in nutrient resource acquisition. Meeting Abstract, International Plasmid Biology.

(7). Agron, P.A., A.M. Erler, P.A. Sobecky and G.L. Anderson. 2002. Establishment of uncharacterized plasmids in *Escherichia coli* by *in vitro* transposition. 102nd General Meeting of the American Society for Microbiology General Meeting.

(8). Agron, P.G., Sobecky, P. and G.L. Anderson. 2002. Establishment of uncharacterized plasmids in *Escherichia coli* by *in vitro* transposition. *FEMS Microbiology Letters* 217:249-254.

(9). Sobecky, P.A. 2002. Approaches to investigating the ecology of plasmids in marine microbial communities. *Plasmid* 48:213-221.

(10). Smalla, K. and P.A. Sobecky. 2002. The prevalence and diversity of mobile genetic elements in bacterial communities of different environmental habitats: insights gained from different methodological approaches. *FEMS Microbiology Ecology* 42:165-175.

(11). Beeson, K.E., D.L. Erdner, C.E. Bagwell, C.R. Lovell, and P.A. Sobecky. 2002. Differentiation of plasmids in marine diazotroph assemblages determined by randomly amplified polymorphic DNA analysis. *Microbiology* 148:179-189.

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14. ABSTRACT

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